The role of stimulating, function-blocking and growth-blocking anti-TSH receptor antibodies (TRAbs) in GD, Hashimoto’s disease and in atrophic thyroiditis

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Anti-TSH receptor auto-antibodies (TRAbs) have several characteristics. They are much more prevalent than auto-antibodies against other membrane receptors, they are pathogenic while other anti-thyroid auto-antibodies, antithyroperoxidase and anti-thyroglobulin, are not, and they are either stimulatory or inhibitory while most of the other anti-receptor antibodies are of the latter category. Looking to the past, it appears that it took a long time for the concept of TRAbs to be fully accepted.

The recent landmark represented by the cloning of the TSH receptor opened new approaches in the study of the effects and actions of TRAbs but clearly much remains to be understood. Figure 1 illustrates the various potential effects of TRAbs. The stimulating effect, as well as the competitive inhibition of the binding of TSH to the receptor, or the blocking effect of TRAbs on the stimulatory action of TSH, can be tested in appropriate bioassays now using the highly specific reagent represented by cell lines transfected with recombinant human receptors or the recombinant protein purified thereof.

So far, the detection of TRAbs using immuno-specific method has not reached routine level yet. The various biological assays lead to the characterisation of stimulating (TSI or TSAb), thyrotropin binding inhibitor (TBI) and blocking TRAbs (TSBAb). TBI activity, the more routinely tested, is usually combined with either stimulating or blocking activity, although the concordance between TBI and the stimulatory or blocking activity may be controversial. In many patients with hyperthyroid Graves’ disease (GD), the presence of variable proportions of both stimulatory and blocking activities may be demonstrated. Also, neutral TRAbs, that is TRAbs without TBI, stimulating or blocking activity, might occur although this is still a point in discussion since de detection of neutral antibody rely on immunological methods still in progress.

Whether or not the action of stimulatory TRAbs is typically TSH-like is still under study. Stimulatory TRAbs activate the cAMP pathway, through receptor interaction, and elicit hyperfunction and cell replication. As shown in the fetal model of transplacental thyroid stimulation, stimulatory TRAbs are responsible for both hyperthyroidism and goitre-genesis. In this model, at variance with what is observed in the patients themselves whose thyroid gland may be variably altered by the autoimmune disease, the concordance between circulating TRAb concentration in the mother’s circulation and the severity of hyperthyroidism and goitre in the fetus is excellent. However, the actions of stimulating TRAbs and TSH show important differences a) the kinetics of adenylyl-cyclase stimulation is not linear for TRAb when it is for TSH, b) monoclonal TRAbs with stimulatory activity are devoid of TBI activity and c) stimulatory activity of TRAb is significantly increased by non ionic hydrophilic polymers such as PEG. Also, the existence of separate epitopes for stimulatory and blocking TRAb on the extracellular domain of the TSH receptor is still a matter of debate. Further elucidation of this point warrants the availability of a large panel of monoclonal human TRAbs necessary for the epitope mapping of the receptor.

In this context, the clinical correlates, hence the clinical usefulness, of the TRAbs has been studied in detail, even though the sensitivity and the specificity of the detection methods are not maximum yet.
TRAB AND GRAVES’ DISEASE

Diagnostic value of TRAb

The prevalence of TRAb at diagnosis of hyperthyroid GD ranges from 70 to 100%. In unselected patients, the titer of TBII, at diagnosis, appears rather low, averaging 30-40% of inhibition of labeled TSH binding. The initial TSAb activity averages 200-300% as expressed in percent increase of basal cAMP production. These relatively low levels of TRAb activity in routine assays are consistent with the result of quantitative studies indicating that the average Graves’ serum contains TRAb concentrations much lower than 1-5 µg/ml when TPO-Ab may reach concentrations of 1mg/ml. With current methods, the proportion of patients with undetectable TRAb before treatment averages 5%.

Because the presence of TRAb is specific for GD, TRAb positivity in patients with nodular or multinodular toxic goiter is indicative of the combination of the two diseases. This occurrence is, however, common only in areas with mild iodine deficiency but can be recognized also in the case of associated suspicion of Graves’ ophthalmopathy.

TRAb as a marker of severity

It is common observation that the level of TRAb only grossly parallels the degree of hyperthyroidism as assessed by the serum levels of thyroid hormones which suggests the existence, in the diseased thyroid of the patients, of alterations capable of modulating the nature or the expression of the interaction of TRAb with thyroid cells. TRAb levels could more likely reflect the intensity or duration of the intrathyroidal inflammatory autoimmune reactions. In general, a good agreement has been observed between TRAb and thyroid volume in untreated patients with GD.

TRAb as an aid to the choice of treatment?

In other words, since the basic therapeutic alternative, for hyperthyroid GD patients, is medical, with a high risk of relapse, or radical/ablative, does the assay of TRAb contribute to the allocation of the patient to the optimum treatment arm? There is no simple answer to that question and it appears that TRAb determination is only one of the indicators to identify patients who are likely to achieve remission without ablative treatment. The initial TRAb status has not had high enough positive and negative predictive values for remission or relapse after completion of antithyroid drug (ATD) treatment to support, by itself, the treatment decision in most patients. However, taking into account the titer of TRAb and other indicators such as the age, gender, thyroid volume, severity of hyperthyroidism and, possibly, presence of ophthalmopathy, subgroups of patients can be identified with a high or low risk of relapse, respectively.

Although it is not necessary for the diagnosis of GD except in some cases of multinodular goiter, initial TRAb measurement is useful as a marker of the disease severity and may, in combination with other clinical indicators, contribute to the treatment decision.
TRAb and the management of ATD treatment

Because recent surveys of treatment policies have shown that non-destructive therapy for hyperthyroid GD remains the preferred modality of treatment in most centers outside North America, optimization of ATD administration remains a valid topic of clinical research in order to find ways to minimize the relapse rate. Many studies of dosage and duration of ATD treatment have been reported.

Studies have found that the rate of fall of TRAb (TBII) on ATD treatment is predictive of subsequent outcome. In Michelangeli et al’s series [20], 73 and 70% TBII-negative, as compared to 28 and 17% TBII-positive patients, at 12 and 18 months of treatment, respectively, remitted. In this study, TBII values measured at 6 months of treatment were not discriminant. However, in Cho et al’s series of 174 patients [3], with an overall rate of remission of 52%, the proportion of remitters was much higher in those patients treated for 24 months who had become TBII-negative with normal basal TSH after 6 (94% remission) or 12 (75% remission) months of treatment, than in the ones in whom TRAb had remained detectable for 18 (63% remission) or 24 months (52% remission). The duration of the ATD course can be adapted to the TRAb status. In Edan et al’s study [6], the 44/64 patients tested every 3 months in whom ATD was stopped when TRAb (TSAb) became negative were maintained on ATD for an average of 9 months (range: 3-18); among them, the relapse rate was 41%, as compared to 92% for the patients who remained TSAb-positive after 18 months of ATD treatment.

The studies investigating TRAb assays at the end of an ATD course of predetermined duration have been analyzed by Feldt-Rasmussen et al. [7]. This analysis clearly shows the value and the limitations of the TRAb predictions. TRAb negative patients had 65% less risk of relapsing than TSHR-Ab positive ones. By increasing the cut-off level for TRAb positivity, it is even possible to obtain a predictive value of a positive test of almost 1.00, but at the expense of lowering the predictive value of a negative test.

Assays for TRAb should not be taken as a “magic tool” for the management of ATD treatment, but just as one indicator among others (thyroid volume, basal TSH,....) in a complex and multifactorial situation. Clearly, the TRAb contribution to the prediction of post-treatment outcome is significant but limited. It is therefore useful in clinical practice when the risks of the wrong therapeutic decision appear significant. More informative appears to be the longitudinal study of TRAb levels rather than a single end-of-treatment determination. It would be appropriate, using the new generation TRAb and possibly epitope-specific assays, to design such prospective clinical studies.

TRAb and radioiodine treatment

Radioiodine efficiency does not appear to be dependent on TRAb status. However, residual hyperthyroidism was significantly correlated with higher pre-treatment TRAb levels. Initial TRAb levels appear to contribute to the thyroid resistance to radioiodine therapy. Radioiodine treatment is associated with a transient increase in the level of TRAb and, in some cases, the appearance of blocking TRAb. To what extent the post-irradiation thyroid function evolution is dependent on changes in TRAb levels or activity is currently under study. Early, transient hypothyroidism might be associated with blocking TRAb or, on the contrary, the recovery of euthyroidism with an increase in stimulating antibodies [25].

TRAb and surgical treatment

TRAb determination has no practical routine usefulness in evaluating surgical treatment except that a) persistence of high levels of TRAb during ATD treatment may contribute to the decision of surgical treatment, b) it has been of interest to study the evolution of circulating TRAb after removal of the thyroid and c) post-surgery outcome of thyroid function is party related to TRAb status. Following surgery, TRAb decline and become undetectable in a majority of patients in 6-9 months. The disappearance of TRAb from the circulation within a few weeks in many patients is in line with the concept of the target organ being the main site of autoantibody production. However, this is not always the case. Whether post-operative TRAb levels correlate with residual thyroid volume has not been studied thoroughly, but it has been suggested that thyroidectomy could modulate immunological activity of the disease.

GD and pregnancy

Feto-neonatal hyperthyroidism is observed in 2-10% of pregnancies in mothers with current or previous GD. This is due to the maternal TRAb. It represents a serious condition, with 16% neonatal mortality, the risk of intrauterine death and stillbirth, and skeletal developmental abnormalities including craniosynostosis. Feto-neonatal hyperthyroidism occurs in association with the highest levels of maternal TRAb [4, 17]. It is therefore a predictable disease. Recently, the following guidelines for measurements of TRAb in pregnancy have been proposed in the American as well as European literature [15, 19]:

— in the case of antecedent GD in remission after ATD treatment, the risk of feto-neonatal hyperthyroidism is negligible and systematic measurement of TRAb is not necessary. However, thyroid function should be evaluated during pregnancy not to over-
look an unlikely but possible recurrence. In that case, TRAb assay is mandatory
— in the case of antecedent GD previously treated with radioiodine or thyroidectomy, and whatever the current thyroid status (euthyroidism with or without thyroxine substitution), TRAb must be measured early in pregnancy to evaluate the risk of fetal hyperthyroidism. If the level is high, careful monitoring of the fetus is mandatory for early detection of signs of thyroid overstimulation (pulse rate >> 170 b/m, impaired growth rate, oligoamnios, goiter). It may be appropriate to consider direct diagnosis in the fetus. Fetal blood sampling through cordocentesis is feasible as early as 25-27 weeks of gestation with less than 1% adverse effects (fetal bleeding, bradycardia, infection, spontaneous abortion, in utero death) in experienced centers. ATD administration to the mother may be considered in order to treat the fetal hyperthyroidism even though the management by such a treatment does not yet benefit from wide experience [22].
— in the case of concurrent hyperthyroid GD, whether it has preceded the onset of pregnancy or not, ATD treatment being adjusted to keep free T4 in the high normal range to prevent fetal hypothyroidism, TRAb should be measured at the beginning of the last trimester, especially if ATD dosage required is high. If TRAb assay is negative or the level low, fetal-neonatal hyperthyroidism is unlikely. If antibody levels are high (TBII ≥ 40 U/l or TSAb ≥ 300%, new TRAK assay > 7 U/l), fetal hyperthyroidism must be considered likely.
— in the case a previous newborn with hyperthyroidism, TRAb assay should be performed early in the course of pregnancy.

When TRAb level is significant during late pregnancy, TRAb must be determined on cord blood in the newborn and then sequentially, at 7-10 day intervals for 2-4 months usually, in order to monitor the duration and dosage of ATD treatment.

Which TRAb assay should be used is a point in discussion. The bioassay for stimulating antibody should theoretically be preferred to the radio-competitive assay. However, there are arguments to take into account:
— only in the near future, when simpler stimulatory bioassays or direct epitope-specific assays are available, will the practical advantages of the current commercial radio-competition assays be overridden.
— in the experience of most authors, TBII serves the purpose of detecting transplacental feto-neonatal hyperthyroidism satisfactorily, the occurrence of natural stimulatory antibodies devoid of activity in the radio-competition assay being exceptional
— from a recent report, it appears that there may be a shift from stimulating to blocking activity of TRAb during pregnancy with a decrease in TSAb in the face of unchanged TBII levels. This fascinating observation should be explored further [13].

We therefore suggest the use of the radio-competition method for routine detection of TRab. The positive sera, a minority, should be tested subsequently in stimulation and blocking bioassays.

**GD in children and adolescents**

Hyperthyroid GD is, on the average, more severe in younger patients so that thyroid ablation ends up, in the long run, as the usual therapeutic option. Long term remission rates, in children and adolescents, are usually less than 30-40% and much lower in prepubertal (17%) than pubertal children (30%). Predictors of early remission include age, BMI, heart rate, goiter size, serum T4 and T3 concentrations, platelet count and TRAb at diagnosis, as well as changes in goiter size and time required for serum T4 and T3 concentration to normalize on ATD treatment [8].

**Extra-thyroidal manifestation of GD**

Graves’ophthalmopathy, pretibial myxedema and acropachy are observed in 60%, 2-5% and less than 1% of patients with GD, respectively. Pretibial myxedema, often observed after radioiodine treatment usually with ophthalmopathy, is always associated with very high titers of TRAb, although the link between the two is not understood. In cases of pretibial myxedema of an unusual type, the assay of TRAb is useful for the confirmation of the diagnosis.

Graves’ophthalmopathy is clearly not caused by anti TRAb as shown in the materno-feto-neonatal model of transplacental hyperthyroidism. However, there is an association between TRAb and Graves’ophthalmopathy in epidemiological as well as longitudinal studies. The demonstration of the expression of the TSHR by activated retroocular fibroblasts could suggest an implication of TRAb or TSHR specific T cells in this disease. The epitopes involved in retroocular autoreactivity could be non-specific or neutral as sera from patients negative for classical TRAb have been shown to react with the TSHR in western blotting [16]. In clinical practice, the detection of TRAb is useful in the case of suspected euthyroid Graves’ophthalmopathy as one of the abnormalities characterizing the subclinical thyroid disease. TRAb are detected in 32-40% of the cases, of euthyroid GD. In some patients, the presence of TRAb may be the only detectable abnormality [14].
**PREVALENCE OF TRAB IN AUTOIMMUNE THYROIDITIS**

The prevalence of TBII ranges from 0 to 44% in goitrous thyroiditis (mean: 9%) and from 0 to 54% (mean: 21%) in atrophic thyroiditis (primay myxedema). For TSBAb, the corresponding figures are 0-44% (mean: 12%) and 0-62% (mean: 33%), respectively [5]. In the report of Cho et al, the prevalence of TBII is 6.3 and 48.0%, and that of TSBAb 10.5 and 59% for goitrous and atrophic thyroiditis, respectively [2]. Clearly, the prevalence of TSBAb is higher in non goitrous than goitrous autoimmune thyroiditis as well as in overt than in subclinical hypothyroidism or euthyroid thyroiditis [1].

**Identification of transient transplacental neonatal hypothyroidism**

The hypothesis of the responsibility of maternally transmitted antithyroid antibodies as a cause of familial congenital hypothyroidism was first proposed by Beierwaltes et al in 1959. Then, it became progressively evident that maternal anti-thyroglobulin and anti-thyroid peroxidase antibodies are not pathogenic to the fetus. In 1980, Matsuura et al reported the first cases of transient neonatal hypothyroidism due to maternal TSBAb [18]. Since then, the prevalence of TBII in newborns with congenital hypothyroidism has been estimated to range between 0.8 and 38%. In the mothers of hypothyroid newborns, TBII is detected in 5% and TSBAb in 4%. On the whole, transient antibody-related neonatal hypothyroidism amounts to 1% of all causes of congenital hypothyroidism. From a practical point of view, present assay methods do not permit the routine systematic detection of TSBAb in neonatal blood spots. However, transient transplacental neonatal hypothyroidism is predictable just as fetoneonatal hyperthyroidism, by the assay of maternal TBII, and if positive, TSBAb, during the last trimester. Epidemiological data suggest that only in women with atrophic primary myxedema is this screening recommended. Of note, a) there is a correlation between the severity of the hypothyroidism as assessed by the thyroid function at birth and the degree of development of the inferior femoral epiphysis and the inhibitory activity of TSBAb, b) thyroid uptake of radioiodine or pertechnetate is suppressed in this condition. Vigorous substitutive treatment of the neonate is mandatory as early as possible, when hypothyroidism has been expected or diagnosed at neo-natal screening. The disappearance of TSBAb has to be monitored at 7-15 day intervals in the newborn but the substitutive treatment is generally maintained for several weeks or months after the 1.5-2 month period when the TSBAb assay has become negative.

**Spontaneous remission from autoimmune hypothyroidism**

In the few series reported, reversibility of autoimmune hypothyroidism may be observed in 0 to 24% of the patients. Is this condition predictable? Takasu et al. found TSBAb in 10% of patients with goitrous autoimmune thyroiditis and 25% of those with the atrophic form [23]. During a maximum follow up period of 11 years, of the 21 patients with blocking antibodies, 15 became negative. Among them, 6 remained euthyroid after thyroid treatment withdrawal. In contrast, in the 6 TSBAb-positive patients with atrophic thyroiditis, blocking antibodies and hypothyroidism persisted for the whole follow up [23]. On the whole, measurement of TSBAb is not a specific marker of spontaneous recovery from hypothyroidism because disappearance of the antibody is not necessarily parallel to that of hypothyroidism.

**TRAb and atypical thyroid autoimmune patterns**

**Fluctuating thyroid function**

Spontaneous evolution of hyperthyroid GD to hypothyroidism has been characterized and, in many cohorts with long term follow up of 10-15 years, occurs in 2,1-2,8% of the patients treated with ATD [9]. Different mechanisms may be involved. While development of destructive autoimmune thyroiditis has been observed, presence of TSBAb is demonstrated in 20-40% of the cases [24]. TSBAb may apparently result from a conversion of the bioactivity of TSAb or coexist with TSAb at the time of hypothyroidism. TBII is usually detectable. In most cases, hypothyroidism is definitive so that the presence of TBII is not, in itself, an indicator of subsequent normalization.

More than 60 cases of fluctuating thyroid function have been reported, most of them with the spontaneous evolution of hypo to hyperthyroidism [5]. In a few cases, cycles of transition from hypo to hyper to hypothyroidism or the converse have been observed [10, 12]. Determination of the TRAb, TBII and stimulating/blocking antibodies, is useful to try and characterize these unusual anecdotal cases although there is not always a concordance between thyroid status and the net bioactivity of TSHR-Ab.

**Hypothyroid GD**

Hypothyroid GD is defined as the development of Graves’ ophthalmopathy in a hypothyroid subject. The association of infiltrative ophthalmopathy with primary hypothyroidism is rare [11]. In this recently reported series of 5 patients, all were positive for TSAb but not TSBAb. Thyroid function was fluctuating in 4 of them. All
had very high levels of antithyroglobulin and antithyroperoxidase antibodies. In these cases, TRAb assay confirms the association of GD and autoimmune thyroiditis.

**Painless thyroiditis**

TRAb are uncommon in post-partum thyroiditis, so that they are not predictable for recovery. In the sporadic forms of painless thyroiditis, TRAb are detected in 6 to 20% of the cases. In some case reports, the determination of TRAb, and sometimes TSBAb, is useful for the diagnosis [21]. In few instances with occurrence of hypothyroidism, the disappearance of TSBAb justifies a substitutive treatment withdrawal trial.

**REFERENCES**